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# Serum biochemical and oxidative status in Holstein cattle affected with foot and mouth disease

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## ABSTRACT

Foot and mouth disease (FMD) is a severe, highly contagious viral disease of cloven-hoofed ruminants caused by an aphthovirus of the family Picornaviridae. The disease in cattle is clinically characterized by fever and vesicles on the foot, in the oral cavity and on the mammary gland.This study was carried out to determine the changes in some serum biochemical parameters of cattle naturally infected with FMD O in Shahrekord district, Iran. For this purpose, blood samples were obtained from 23 Holsteins with clinical signs of FMD, as well as 22 blood samples from healthy animals. Serum analysis revealed significantly higher levels of AST, CK, CK-MB and LDH activities as well as MDA, troponin I, glucose and triglycerides concentrations in FMD-affected cattle compared to healthy control group ($p < 0.05$). Serum GPx and SOD activities in cattle with FMD were significantly lower than those in normal animals ($p < 0.05$), while there was no significant difference in serum CAT activity between 2 groups of animals. It is concluded that oxidative stress and some degrees of myocardial and pancreatic lesions develop in FMD-affected cattle. These findings provided information to better understand the pathogenesis of the disease and gives further insight to improve supportive treatment procedures in FMD virus infection in cattle.

## Keywords

*Foot and mouth disease, cattle, Picornaviridae, serum biochemistry, pancreas*

## Abbreviations

FMD: Foot and mouth disease
AST: Aspartate aminotransferase
CK: Ceatinine Kinase
CK-MB: Creatinine kinase myocardial band
LDH: Lactate dehydrogenase
MDA: Malondialdehyde
SOD: Superoxide dismutase
GPx: Glutathione peroxidase



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## Introduction

FMD is a severe, highly contagious viral disease of cloven-hoofed ruminants. The causative agent of FMD is an *aphthovirus* of the family *Picornaviridae* with seven strains (A, O, C, SAT1, SAT2, SAT3, and Asia1) which are endemic in different countries worldwide including several parts of Asia and in most of Africa and the Middle East [1].The causative agent can be found in all secretions and excretions from acutely infected animals and spreads rapidly by various direct and indirect contacts and airborne routes. FMD in cattle is clinically characterized by high fever, profuse salivation, and vesicles at the interdigital cleft in the oral cavity and on the mammary gland [2]. The disease is rarely fatal in adult animals, but there is often high mortality, up to 50%, in young animals due to myocarditis [3].

There have been few studies conducted on pathogenesis of FMD in domestic animals [4,5]. These studies generally conducted on virus kinetics in the host rather than the development of lesions in tissues and following organ failure. On the other hands , much of the basic knowledge about FMDV virus–host interactions is derived from *in vitro* studies under controlled cell culture conditions. These studies, while important, cannot fully address the complexity of the virus–host interaction at the tissue, organ and systemic levels in the natural hosts[6]. Additionally, there is limited information in the literature about the serum biochemical findings of cattle naturally infected with the FMD virus [7]. Serum biochemical analysis can be a useful tool for assessing animal health and helps better understanding the pathogenesis of the disease. Therefore, the aim of this study was to determine biochemical and oxidant-antioxidant status by evaluating some oxidative stress parameters and serum biochemical profile in Holstein cattle naturally affected with FMD.

## Results

FMDV partial gene sequence was detected using RT-PCR test specific for VP1 nucleotide fragment. The FMDV-specific band with the size of 108 bp was detected in the tested sample and FMDV positive control. The positive PCR product band was the same as the positive control. No band was observed in the negative control (Fig. 1).

After sequencing of the RT- PCR products and preforming BLAST analysis, the FMDV VP1 gene fragment sequence was confirmed. Furthermore, alignment of the read sequences with the published sequences in the NCBI gene bank confirmed the presence of FMDV serotype O in the sample (99.8% identity).

The serum activities of CK, CK-MB, AST and LDH of FMD-affected cattle were significantly higher than healthy animals ($p < 0.001$) (Table 1). Serum amylase and lipase activities of FMD-affected cattle were also significantly higher than those in normal animals ($p < 0.05$) (Table 2). Serum concentration of troponin I, glucose and triglycerides of FMD-affected cattle were significantly elevated in comparison to healthy animals ($p < 0.05$) (Tables 1 and 2). The result of oxidative stress indices measurement is shown in Table 3. Serum activities of SOD and GPx of FMD-affected cattle were significantly lower than those in healthy animals ($p < 0.05$), while serum catalase activity showed no significant difference between FMD-affected and healthy cattle ($p > 0.05$). The serum concentration of MDA of FMD-affected cattle was significantly higher than that in the control group ($p < 0.001$) (Table 3).

## Discussion

Serum biochemical references of cattle naturally infected with FMD virus are not known. The results of the present study revealed that serum activities of CK and AST, biomarkers of muscle degeneration and necrosis, in cattle with clinical FMD were increased and were significantly higher than those of healthy cattle. Furthermore, serum CK-MB activity and concentration of troponin I, biomarkers of myocardial damage, in cattle with FMD were also significantly higher than those in normal animals. Myocarditis with high mortality in neonate calves and lambs is a well known feature of FMD [1]. It has been suggested that after initial replication of FMD virus in the oropharynx, it disseminates to the secondary sites including cardiac muscle in suckling ruminants, and virus replication in this tissue results in cardiac degeneration and necrosis [8]. Following cardiac cell damage, CK-MB and troponin I is released to the blood stream and their serum levels elevates, as indicators of myocarditis [9,10].

AST is present in all tissues except bone, with the highest levels in liver and skeletal muscle. CK activity is also greatest in skeletal muscle and rapidly increases in serum following muscle injuries. On the other hand, serum AST activity in cows with muscle injury slowly increases and has longer half-life than of CK [11]. AST is also present in high levels in the hepatocytes and its serum elevation is routinely considered as a sensitive indicator of hepatocyte damage, even if the damage is of a subclinical nature [12]. However, concurrent serum elevation of AST with CK is considered as inthe dicators of muscular damage [13]. The results

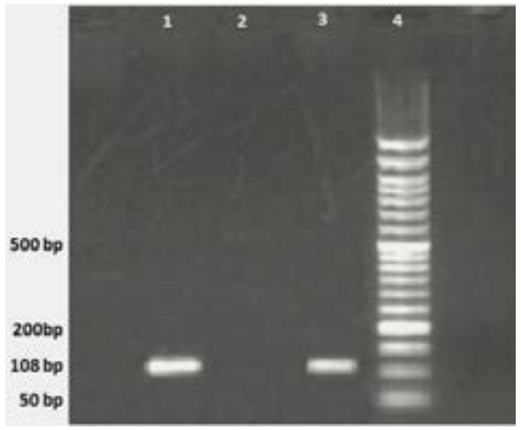


**Figure 1**
Electrophoresis of RT-PCR products. lane 1: positive control (108 bp). Lane 2: negative control. Lane 3: positive sample (108 bp). Lane 4: ExcelBand™ 50 bp DNA ladder.

**Table 1.**
Parameters associated with cardiac and skeletal muscle damage in the FMD group and control group. Data are expressed as mean and standard error of the mean (Mean ± SE)

| Parameters | FMD | Control | *p* |
|---|---|---|---|
| CK (U/L) | 267.40 ± 11.01 | 140.37 ± 8.47 | 0.001 |
| CK-MB (U/L) | 127.86 ± 6.78 | 71.73 ±3.28 | 0.001 |
| AST (U/L) | 54.50 ± 3.85 | 31.00 ±1.02 | 0.001 |
| LDH (U/L) | 1484.58 ± 72.67 | 709.26 ± 42.53 | 0.001 |
| Troponin I (ng/mL) | 0.020 ± 0.0013 | 0.016 ± 0.0006 | 0.033 |

**Table 2.**
Parameters associated with pancreatic damage in the FMD group and control group. Data are expressed as Mean ± SE

| Parameters | FMD | Control | *p* |
|---|---|---|---|
| Amylase (U/L) | 81.37 ± 2.50 | 52.70 ± 2.32 | 0.001 |
| Lipase (U/L) | 39.00 ± 2.03 | 33.26 ± 1.60 | 0.031 |
| Glucose (mg/dL) | 73.73 ± 12.57 | 44.33 ± 11.59 | 0.001 |
| Triglycerides (mg/dL) | 22.76 ± 1.53 | 16.33 ± 1.08 | 0.033 |

**Table 3.**
Parameters of oxidative status in the FMD group and control group. Data are expressed as Mean ± SE

| Parameters | FMD | Control | *p* |
|---|---|---|---|
| MDA (ng/mL) | 4.58 ± 0.24 | 2.27 ± 0.14 | 0.001 |
| GPx (U/L) | 11.70 ± 0.04 | 14.52 ± 0.77 | 0.002 |
| CAT (Ku/L) | 1.21± 0.04 | 1.18 ± 0.20 | 0.323 |
| SOD (% inhibition) | 27.53 ± 1.23 | 30.79 ± 1.37 | 0.047 |

of the present study showed that during the course of FMD in non-suckling cattle, some degrees of injuries occurs in cardiac and skeletal muscles. Although the myocardial degeneration and necrosis and associated mortality is a common finding in the suckling calves, lambs and pigs, the myotropism and the mechanism of muscular damage of FMDV are not fully understood. LDH is an enzyme involved in energy production that is found in almost all cells of the body, with the highest levels found in the cells of the heart, liver, muscles, kidneys, lungs, and in blood cells [12]. Although determination of serum LDH activity is one of the most frequently performed assays as an aid in the diagnosis of myocardial and pulmonary infarction, it suffers from lack of specificity for cardiac disease [1]. Thus, serum elevation of LDH as was observed in the cattle affected by FMD indicates the involvement of various tissues. Elevation of serum CK-MB, AST and LDH activities have been reported in buffaloes infected with FMD virus, serotype O [14]. High levels of serum troponin I concentration has also been detected in calves and lambs suffered from FMD [9,10]. Although significantly higher levels of aforementioned parameters in cases of FMD in comparison to control animals is noticeable, and shows some degrees of myocardial damage, but its clinical importance is unknown for authors. Very high levels of serum CK and troponin I has been reported in clinical cases of FMD in lambs and calves [9, 10].

The present study demonstrated that FMD in cattle increases lipid peroxidation end products which was indicated by the elevation of the MDA concentration in the serum of affected animals compared to the healthy subjects. Polyunsaturated lipids are susceptible substrates to free radical oxidative damage and biomarkers of lipid peroxidation including MDA, are considered as a reliable marker for oxidative stress [15].This study revealed decreased activities of SOD and GPx in FMD-affected cattle compared with the control group. There were also no significant differences between the mean activities of CAT in the control group compared with the infected group. These are in agreement with the findings of Khoshvaghti et al. (2014) that reported low serum activities of SOD and GPx with no changes in CAT in FMD cattle infected with FMD virus [7]. SOD, CAT and GPX are the first line defense antioxidant enzymes. These enzymes dismutate superoxide radicals, breakdown hydrogen peroxides, and hydroperoxides to harmless molecules (H2O2/alcohol and O2), respectively [16]. It has been suggested that lower antioxidant enzyme activity might be caused by the depletion of antioxidant defense system occurring as the consequence of overproduction of free radicals induced by various factors, including virus infections [17]. During oxidative stress, production of highly reactive oxygen species (ROS) beyond the scavenging capacity of antioxidant defense mechanisms is directly involved in the oxidative damage of macromolecules including lipids, proteins and nucleic acids in tissues. Accumulating studies have indicated that excessive ROS production plays important roles in the pathogenesis of inflammatory diseases. For example, ROS contributes to virus replication and the activation of inflammatory cytokines during the infection of both influenza virus and bovine herpes virus -1 [18].

In the present study serum amylase and lipase activity in the FMD-affected cattle were found to be higher in comparison to healthy animals. Increased serum amylase and lipase activity is used as a reliable biomarker for the diagnosis of acute pancreatitis in animals (Hoffman and Solter2008). Serum activity of these enzymes increases due to the leakage from the inflamed pancreas into the blood stream. Amylase and lipase have relatively short blood half-time and so when rise rapidly after pancreas damage, return to normal range within five days [19]. On the other hand, high serum concentrations of glucose and triglycerides in the FMD-affected cattle in parallel to high serum activities of amylase and lipase found in the present study indicate pancreatic injuries [12] cause by FMD virus infection. Pancreatic acinar necrosis, inflammation and regeneration are manifestations of the acute form of FMD and diabetes mellitus has been observed in both experimental and natural cases of the disease [20]. Pancreatic degeneration and necrosis has been reported in a gazelle naturally infected by FMD virus serotype O1 [21]. It has also been suggested that viral replication in the myocardium and pancreas, and their associated pathologies are two common FMD virus infection features in the mouse model [22]

Enough data is not available for the serum biochemistry of FMD affected cattle. As a conclusion, serum analysis elucidates oxidative stress and some degrees of myocardial and pancreatic lesions in FMD-affected cattle. These findings provided information to better understand the pathogenesis of the disease and gives further insight to improve supportive treatment procedures in FMD virus infection in cattle.

## Material and methods

During an outbreak of FMD in the Shahrekord district, blood samples (10 mL) were obtained from affected 23 cattle by jugular venipuncture using vacutainer tubes. Sampled cattle were Holstein and aged between 2 and 19 months and of both sexes (7 males and 16 females). During the investigation the disease was observed within that age range. Blood samples without anticoagulant were also taken from healthy cattle ( = 21; Holstein bred and age and herd management similar to the first group) originating from a control farm where FMD was not reported. Blood samples were left to clot, centrifugated at 3000 r.p.m for 20 minutes and separated serum samples were stored at -70 °C until biochemical analysis.

Samples including vesicular fluids were collected from the cattle showed clinical signs of FMD. These samples were placed in glycerol saline and kept at -20 °C until subjected to trials of virus detection. RNA was isolated using QIAzol (QIAGEN, Germany, Catalog Number: 79306) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed with TaqMan Reverse Transcription kit (Invitrogen, Germany, Catalog Number: 8080234N) according to the manufacturer's instructions. PCR was carried out using the primers designed for a part of VP1 gene of FMDV using GenScript Primer Design online tool. The sequences of primers were as follows: FMDVF: 5'-TGAGTGCAGGTACAGCAGAA-3' and FMDVR: 5'-ATGGCACCGTAGTTGAAGGA-3'. The PCR thermal cycle reactions consisted of denaturation at 95 °C for 30 s followed by 30 cycles at 95 °C for 20 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 5 minutes. The positive and negative controls prepared from Razi Vaccine and Serum Research Institute was included in each test. Six µL of the amplified products were loaded on a 1.3% agarose gel; visualized by staining with Green viewer (company, country) and compared to DNA markers (Excel Band™ 50 bp DNA ladder, SMOBIO, Taiwan). PCR-positive samples in a volume of 50 µL was sent to the Bioneer Company for sequencing. The sequencing procedure was performed on the ABI 3730XL DNA Analyzer, with a high quality of sequence analysis data (Phred Score (QV): ≥ 20, Guaranteed read lengths: ≥ 600 bp).

Serum activities of aspartate aminotransferase (AST), creatinine Kinase (CK), creatinine kinase myocardial band (CK-MB) and lactate dehydrogenase (LDH), amylase and lipase, and serum concentration of glucose and triglycerides were measured by an auto-analyzer (BT 1500, Italy) using standard diagnostic kits (Dialab, Austria). Serum troponin I concentration were determined by an enzyme-linked immune-absorbent assay (ELISA) kit (Monobind. Inc, Canada). The kit had a detection sensitivity limit of 50 pg/mL troponin I. The intra and inter-assay coefficients of variations were 0.70% and 1.00% for CK, for CK- MB 0.61% and 0.53%, for AST 3.06% and 1.38%, for LDH 2.01% and 2.30% for glucose 1.74% and 1.19%, for lipase 1.01% and 0.82%, for amylase 067% and 0.97%, for triglyceride 1.82% and 1.60%, for HDL 0.69% and 0.58%; and for cholesterol 0.61% and 1.22%, respectively.

Serum malondialdehyde (MD) concentrations, also known as thiobarbituric acid reactive substances (TBARS), were determined colorimetrically using Buege & Aust method (1978)[23]. The activity of superoxide dismutase (SOD) was measured by nitrobluetetrazolium (NBT). In this method the superoxide radicals change NBT to blue NBTH2. By adding the serum to the studied material, producing of blue color by superoxide dismutase is controlled [24]. Catalase (CAT) activity was determined by using the Goth method (1991)[25]. Glutathione peroxidase was determined using the method described by Paglia and Valentine (1967)[26].

### *Statistical analysis*

All biochemical data were expressed as the mean ± standard error of the mean. Differences between the groups were tested by *t*- test. Statistical analysis was performed by using Sigma Plot 12 software. A *p*-value of less than 0.05 was considered statistically significant.

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## Author Contributions

All authors contributed to the design of study, data analysis and manuscript preparation.

## Conflict of Interest

The authors declare that there is no conflict of interest.

## References

1. Constable PD, Hinchcliff KW, Done SH, et al.Veterinary Medicine, A Textbook of Diseases of Cattle, Horses. Sheep, Pigs and Goats. 11 ed. St. Louis: Elsevier, 2017;pp.
2. Poonsuk K, Giménez-Lirola L, Zimmerman JJ. A review of foot-and-mouth disease virus (FMDV) testing in livestock with an emphasis on the use of alternative diagnostic specimens. Animal health research reviews 2018;19:100-112.
3. Admassu B, Getnet K, Shite A, et al. Review on foot and mouth disease: Distribution and economic significance. Academic Journal of Animal Diseases 2015;4:160-169.
4. Arzt J, Juleff N, Zhang Z, et al. The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. Transboundary and emerging diseases 2011;58:291-304.
5. Stenfeldt C, Segundo D-S, de los Santos T, et al. The pathogenesis of foot-and-mouth disease in pigs. Frontiers in veterinary science 2016;3:41.
6. Arzt J, Baxt B, Grubman M, et al. The Pathogenesis of Foot-and-Mouth Disease II: Viral Pathways in Swine, Small Ruminants, and Wildlife; Myotropism, Chronic Syndromes, and Molecular Virus–Host Interactions. Transboundary and emerging diseases 2011;58:305-326.
7. Khoshvaghti A, Askari A, Nazifi S, et al. Evaluation of some antioxidant enzymes in cattle infected with foot and mouth virus. İstanbul Üniversitesi Veteriner Fakültesi Dergisi 2014;40:70-75.
8. Ryan E, Horsington J, Durand S, et al. Foot-and-mouth disease virus infection in young lambs: pathogenesis and tissue tropism. Veterinary microbiology 2008;127:258-274.
9. Aslani MR, Mohri M, Movassaghi AR. Serum troponin I as an indicator of myocarditis in lambs affected with foot and mouth disease. Veterinary research forum: an international quarterly journal 2013;59.
10. Sobhy NM, Bayoumi YH, Mor SK, et al. Outbreaks of foot and mouth disease in Egypt: Molecular epidemiology, evolution and cardiac biomarkers prognostic significance. International journal of veterinary science and medicine 2018;6:22-30.
11. Weber J, Zenker M, Köller G, et al. Clinical chemistry investigations in recumbent and healthy German Holstein cows after the fifth day in milk. Journal of Veterinary Research 2019.
12. Hoffmann WE, Solter PF. Clinical biochemistry of domestic animals In: Kaneko JJ, Harvey JW,Bruss ML, eds. 6 ed: Academic press, 2008;pp.

13. Burns L, Ramos A, Veiga A, et al. Evaluation of muscle tissue and liver glycogen of cattle submitted to transport over long distances and subjected to emergency slaughter. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2019;71:1067-1075.

14. EL-Deen NAMN, Neamat-Allah AN, Rizk LG, et al. Serological, hematological, biochemical and oxidative markers during foot and mouth disease serotype 'O'infection, Egypt. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Veterinary Medicine 2017;74:218-226.

15. Singh Z, Karthigesu IP, Singh P, et al. Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: a review. Iranian Journal of Public Health 2014;43:7-16.

16. Ighodaro O, Akinloye O. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine 2018;54:287-293.

17. Strycharz-Dudziak M, Kiełczykowska M, Drop B, et al. Total Antioxidant Status (TAS), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) in Oropharyngeal Cancer Associated with EBV Infection. Oxidative medicine and cellular longevity 2019;2019.

18. Ye S, Lowther S, Stambas J. Inhibition of reactive oxygen species production ameliorates inflammation induced by influenza A viruses via upregulation of SOCS1 and SOCS3. Journal of virology 2015;89:2672-2683.

19. Zendehbad B, Alipour A, Zendehbad H. Effect of tetracycline administration on serum amylase activity in calves. SpringerPlus 2013;2:330.

20. Barker I, Van Dreumel A. Foot-and-mouth disease In: Jubb KVF, Kennedy PC,Palmer N, eds. Pathology of Domestic Animals: Academic Press, San Diego, 1992;pp: 141–144.

21. Berkowitz A, Waner T, King R, et al. Description of the pathology of a gazelle that died during a major outbreak of foot-and-mouth disease in Israel: clinical communication. Journal of the South African Veterinary Association 2012.

22. Habiela M, Seago J, Perez-Martin E, et al. Laboratory animal models to study foot-and-mouth disease: a review with emphasis on natural and vaccine-induced immunity. The Journal of general virology 2014;95:2329.

23. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods in enzymology: Elsevier, 1978;pp: 302-310.

24. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clinical chemistry 1988;34:497-500.

25. Goth L. A simple method for determination of serum catalase activity and revision of reference range. Clinica chimica acta 1991;196:143-151.

26. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine 1967;70:158-169.